

OXYGEN UPTAKE OF THE SOLITARY TUNICATE *STYELA PLICATA*

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The solitary tunicate, *Styela plicata*, reaches its northern-most occurrence on the east coast of North America in the vicinity of Cape Hatteras, North Carolina (Van Name, 1945). The cape is an important biogeographic boundary caused by abrupt latitudinal differences in water temperature as a result of the eastward movement of the Gulf Stream (Cerame-Vivas and Gray, 1966). Further northward colonization of *Styela* might be limited by the effects of low winter temperatures on adult survival or by summer temperatures suboptimal for reproduction (Kinne, 1963).

Just south of Cape Hatteras in the Newport River estuary, North Carolina, larval settlement of *Styela* occurs mainly during spring and fall, when water temperature is approximately 20°C; little or no settlement occurs in summer (26-30°C) or winter (5-15°C) (Sutherland and Karlson, unpublished). Similar observations on settlement of *Styela* have been made in Japanese waters (Kazihara, 1964). Since summer temperatures in excess of 20°C are common at least as far north as Cape May, New Jersey (Anon., 1954), the northern extension of *Styela plicata* may be limited by winter mortality.

In an attempt to obtain a physiological explanation of the reduced rate of larval recruitment of *Styela plicata* in the summer in the Newport River estuary, I hypothesized that the metabolic costs of routine body functions require most of the food material absorbed in the gut at the high summer temperatures (26-30°C), thus limiting reproduction, and that only during the intermediate temperatures associated with the spring and fall (20°C) was a surplus of absorbed material available for growth or reproduction.

As part of a larger study designed to test the hypothesis described above, I have measured the routine oxygen consumption of *Styela plicata* in order to estimate the metabolic costs of routine body functions. Oxygen uptake was measured as a function of body size and temperature. In addition, data on the relationship between oxygen tension and oxygen consumption were obtained to provide information on the dynamics of gas exchange in the branchial sac, the gas exchange organ of tunicates.

MATERIALS AND METHODS

At seasonally defined intervals during a two year period when water temperature had not fluctuated more than a few degrees for at least one week, specimens of *Styela plicata* were collected and transferred to an aquarium supplied with running water from the estuary. The aquarium was maintained at the temperature of the estuary at the time of collection ($\pm 1^\circ\text{C}$) and on a light-dark cycle appropriate to the time of year. Five days were allowed for adjustment to laboratory conditions, and animals were discarded at the end of one month.

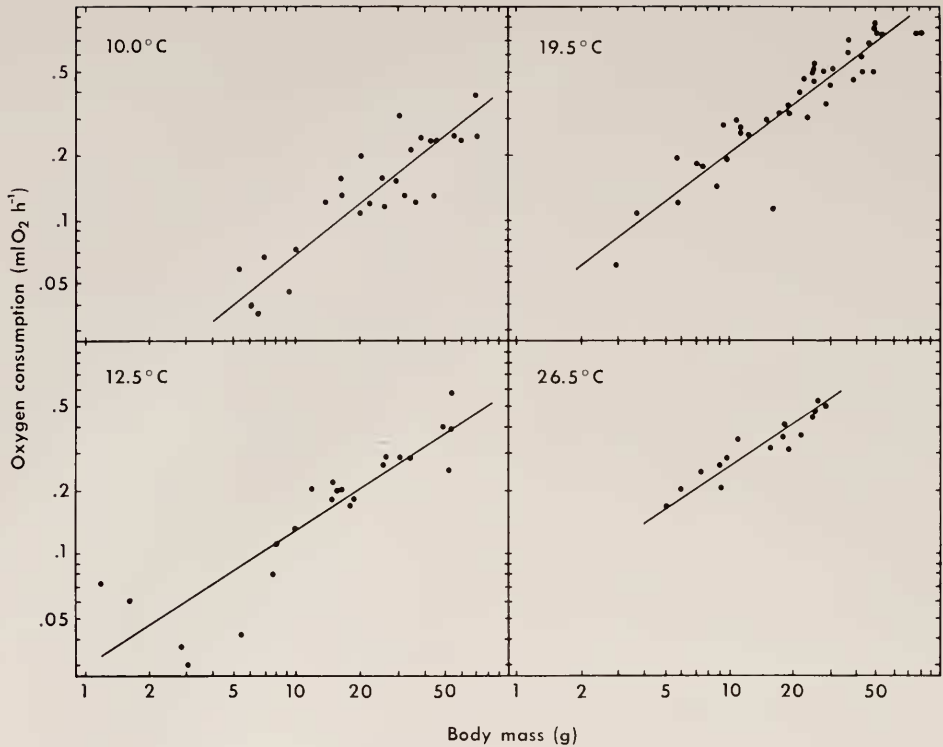


FIGURE 1 The routine oxygen consumption of *Styela plicata* as a function of body mass at four representative temperatures. The slopes of the lines are significantly greater than zero, independent of temperature, and average 0.7.

The experimental chamber for measuring respiration consisted of a 400 cc plastic beaker stoppered by a large rubber cork. Oxygen tension was measured with a YSI oxygen macro-electrode, the linearity of which was checked daily with gas standards. The polarizing circuitry was a design modified after LeFevre, Wyssbrod, and Brodsky (1970) with an output to a Beckman ten inch continuous recorder (model 1005). The oxygen electrode inserted into a hole near the bottom of the beaker, and a magnetic stirring bar maintained water flow past the electrode and provided mixing. A stainless steel screen kept the animal above the stirring magnet and prevented strong currents from developing within the chamber. Once assembled, the entire chamber was placed in a water bath maintained at the experimental temperature ($\pm 0.05^\circ \text{C}$), and recording of the oxygen tension began.

Initially, the chamber was assembled with filtered sea water (Gelman GF/A) but without an animal, and a blank rate of decrease of oxygen tension recorded. The animal was then introduced and the rate of decrease again measured. The first fifteen minutes following introduction of the animal reflected the gradual return of routine activity (as determined by siphon extension), whereupon a linear decrease in P_{O_2} began, continuing until the P_{O_2} in the chamber fell well below normoxic or air saturated conditions. At the critical P_{O_2} , (P_c), the decrease in

oxygen tension became curvilinear (*i.e.*, O_2 uptake dependent on P_{O_2}), usually at a P_{O_2} well below air saturation (hypoxic conditions). The experiment was terminated when sufficient data on the rate of P_{O_2} decrease had been obtained, usually one to two hours after introduction of the animal.

The oxygen consumption (\dot{V}_{O_2}) of the animal was calculated from the normoxic rate of P_{O_2} decrease (estimated from the recording with a straight edge), the volume of the container, and the oxygen solubility. Measurements of \dot{V}_{O_2} were taken without regard to time of day, although most occurred between 0800 and 1800 hours. Salinity of the estuarine water used in the experiments varied between 32 and 36‰.

An estimate of the error involved in determining the rate of P_{O_2} decrease was obtained by xeroxing a record and calculating \dot{V}_{O_2} for each copy. The coefficient of variation ($s_x/\bar{x} \times 100$) was 5% ($\bar{x} = 0.158$ STPD ml O_2 /hr, $s_x = 0.008$, $n_x = 10$; animal wet wt = 25.4 g, temperature = 10° C). An estimate of total experimental variation was obtained by using the standard deviation of the points about the calculated regression line ($s_{y/x}$) for all data at 10° C. A coefficient of variation of 11% was calculated for the above \bar{x} (0.158). The difference between the two estimates is the result of systematic electrode drift during an experiment (probably very small), and variations in animal activity between experiments.

The effect of body mass (M) on oxygen consumption (\dot{V}_{O_2}) was analyzed by means of the allometric equation: $\log \dot{V}_{O_2} = \log K_{O_2} + b \log M$, which is equivalent to: $\dot{V}_{O_2} = K_{O_2} M^b$, where K_{O_2} is the oxygen consumption of an organism of unit mass (one gram). The scaling of metabolic rate to body size is then determined by the exponent of mass, the slope of the log-log plot (b).

RESULTS

The rate of oxygen consumption of *Styela plicata* is shown in Figure 1 at four representative temperatures and is approximately the same order of magnitude (0.1–1.0 ml O_2 /hr) as that reported by Jørgensen (1952) for the tunicates *Molgula manhattensis*, *Ciona intestinalis*, and the bivalve *Ostrea virginica*. The slopes of the lines for data at each temperature (Table I) are significantly greater than zero ($P < 0.05$), but do not have a significant regression with temperature and average 0.7 (s.d. = 0.1). This value is not significantly different from $\frac{3}{4}$, but is significantly different from 1. Thus the intraspecific relationship between oxygen consumption and body size of *Styela plicata* is comparable to the interspecific data reviewed by Hemmingsen (1960).

The unit mass oxygen consumption [K_{O_2} , STPD ml O_2 /(hr·g)] is a convenient reference point in the log-log graph of oxygen consumption versus body size. However, since K_{O_2} is well to the left of most of the data points in each of the graphs in Figure 1, it is very sensitive to the fluctuations of the slope, b , about its average value of 0.7. Therefore, the common slope, $b = 0.7$, was used to calculate K_{O_2} to reduce scatter.

Unit mass oxygen consumption, K_{O_2} , increases with temperature as shown in Figure 2. A highly significant linear regression exists between K_{O_2} and the logarithm of temperature (T): $K_{O_2} = -0.059 + 0.077 \log_{10} T$ ($r = 0.99$). The above equation may be combined with the equation relating oxygen consumption to body mass: $\dot{V}_{O_2} = (-0.059 + 0.077 \log_{10} T) M^{0.7}$. This combined result ex-

TABLE I

Parameters of the allometric equation $\dot{V}_{O_2} = K_{O_2} M^b$, where \dot{V}_{O_2} is oxygen consumption (STPD ml O_2 /hr) of acclimatized *Styela plicata* of mass M (g), K_{O_2} is the oxygen consumption of an individual of unit mass [STPD ml O_2 /(hr·g)], b is the slope of the log-log plot of the above equation, and n is the number of individuals measured.

Temperature	K_{O_2}	b	n	K_{O_2} calculated for $b = 0.7$
6.0	—	—	3	0.0051
10.0	0.0230	0.556	11	0.0146
12.5	0.0293	0.649	23	0.0256
15.0	0.0275	0.751	13	0.0308
19.5	0.0348	0.769	41	0.0427
26.5	0.0536	0.670	16	0.0498
28.6	0.0567	0.829	23	0.0532
32.0	—	—	4	0.061
10/19.5	0.00859	0.879	18	0.0145
28.6/19.5	0.0560	0.747	20	0.0631

presses the predicted routine oxygen consumption as a function of body size and temperature and may be used to estimate routine metabolic costs in the testing of energetic hypotheses.

Since the scaling factor, b , does not have a significant regression with temperature, Q_{10} is independent of body size and can be calculated from the regression equation relating K_{O_2} , the unit mass oxygen consumption, to log temperature: $Q_{10} = [K_{O_2} + 10 (dK_{O_2}/dt)]/K_{O_2} = 1 + (10/K_{O_2}) \cdot (dK_{O_2}/dt)$. This equation was used to calculate Q_{10} since detailed "R-T" information was available; it is a more accurate estimator than using $Q_{10} = (R_1/R_2)^{10/(T_1-T_2)}$, which merely estimates the slope of the line connecting two points.

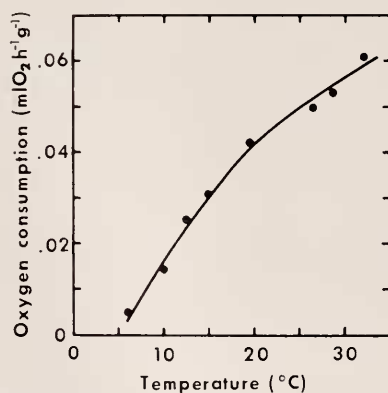


FIGURE 2. The unit mass oxygen consumption, K_{O_2} , of *Styela plicata* as a function of temperature. K_{O_2} was calculated from data such as that presented in Figure 1 for each temperature assuming a slope, $b=0.7$. K_{O_2} has a significant regression with the logarithm of temperature, $K_{O_2} = -0.059 + 0.077 \log_{10} T$ ($r = 0.99$), and the equation is represented by the line.

TABLE II

Statistics for the relationship between critical oxygen tension, P_c , and body mass, M . P_c declines slightly with increasing body size, indicating an increasing capacity to regulate oxygen consumption.

Temperature	Equation	Correlation coefficient (4)	Significance of (r)
12.5	$P_c = 138 - 0.64 M$	-0.98	0.99
15.0	$P_c = 129 - 0.41 M$	-0.85	0.95
19.5	$P_c = 121 - 0.48 M$	-0.67	0.98
18.6	$P_c = 116 - 0.69 M$	-0.65	0.92

For the oxygen consumption of *Styela plicata* acclimatized to temperatures from 10° to 20° C, Q_{10} ranges from 5 to 2 and averages 3. However, between 20° and 30° C, Q_{10} declines to 1.5 and averages 1.7, indicating a relative temperature insensitivity and minimally increased metabolic costs for individuals acclimatized to summer temperatures.

Partial temperature independence might be expected as a result of acclimatization. To test this possibility, the oxygen consumption of *Styela* acclimatized to 19.5° C was measured at 28.6° C and 10° C in acute experiments with immediate transfer from 19.5° C to the experimental temperature. The transferred animals displayed a stable V_{O_2} after approximately one half hour. The data are presented in Table I. There are no significant differences between the acute and acclimatized rates at 28.6° C and 10° C, although the acute rate is 15% higher than the acclimatized rate at 28.6° C. The data suggest some acclimatization to summer temperatures (28.6° C), but the magnitude of the change is small and statistically undetectable.

The effect of oxygen tension upon oxygen uptake can be summarized by means of the critical oxygen tension, *i.e.*, the partial pressure of oxygen at which uptake becomes dependent on P_{O_2} . Larger individuals of *Styela plicata* have lower critical oxygen tensions (Table II) and therefore better regulation of oxygen consumption.

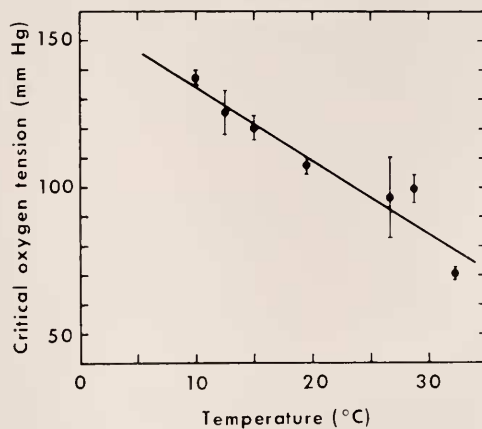


FIGURE 3. The critical oxygen tension, P_c , of *Styela plicata* as a function of temperature. The bars represent one standard deviation, and the slope of the regression equation $P_c = 157 - 2.5T$, is significantly less than zero. Above 10° C, P_c is well below air saturation.

Bayne (1971) and Taylor and Brand (1975) also report an inverse relationship between body size and critical P_{O_2} for several molluscs. The greater effect of temperature on critical oxygen tension is shown in Figure 3, which demonstrates that *Styela plicata* is a better oxygen regulator at higher temperatures.

DISCUSSION

The hypothesis that disproportionately large maintenance costs preclude reproduction by *Styela plicata* at summer temperatures (26–30° C) is evaluated elsewhere (Fisher, 1976). The hypothesis has been shown to be inconsistent with the observed metabolic maintenance costs as indicated by oxygen consumption. Because ingestion rate and oxygen consumption show parallel scaling to body size and temperature, metabolic costs of *Styela* require a constant fraction of the resources available, equivalent to approximately 18% of the organic carbon absorbed in the gut at all temperatures. Thus, only a small and constant fraction of the carbon resources are required for maintenance, and a surplus is available for gonadal and somatic growth over the temperature range 10–30° C. During summer months predation on eggs and newly settled adults and reduction of the adult population appears to be responsible for the observed reduction in larval settlement (Fisher, 1976).

The temperature response of the oxygen consumption of *Styela* (Figure 2) reflects its geographic distribution. Between 20° and 30° C the low Q_{10} and well-developed oxygen regulatory capabilities (Fig. 3) suggest a degree of homeostasis in subtropical environments, in which *Styela* commonly occurs. In contrast, at temperatures below 10° C, *Styela plicata* becomes an oxygen conformer ($P_e \sim$ air saturation), and Q_{10} is very high (> 5). Extrapolation of the data in Figure 2 suggests that aerobic metabolism of *Styela* ceases near 5° C. However, it is likely that the temperature response of metabolic rate is sigmoid, as has been observed for other marine invertebrates (Newell, 1970). It has been possible to maintain *Styela* in the laboratory for several weeks at temperatures as low as 2–3° C, but no pumping or squirting behavior was observed. Furthermore, at temperatures below 8° C, no growth or attachment of *Styela* to its container occurred, although *Styela* grew and attached to substrate in the aquaria at all higher temperatures. These observations suggest reduced physiological adaptation of *Styela* at temperatures below 10° C. Sabbadin (1957) has reported winter mortality of *Styela plicata* in the lagoon of Venice at 4–6° C.

As discussed above, since summer temperatures suitable for reproduction occur at least as far north as Cape May, winter mortality is likely to be the major cause for the absence of *Styela* in areas north of Cape Hatteras. In the Newport River estuary, just south of Cape Hatteras, water temperature during winter months averages 10° C, and occasionally drops below 8° C during unusually cold weather, particularly at low tide. However, high tide usually brings warmer neritic water into this shallow estuary, and such low temperature conditions rarely persist for more than a few hours (temperature records, DUMI museum). In contrast, winter temperatures persisting at or below 8° C are common north of Cape Hatteras and may be responsible for the absence of *Styela plicata* in these areas.

Although little mortality was observed in the running seawater aquaria, the animals maintained below 8° C showed little activity, no growth, and, in the field,

would be very vulnerable to removal from substrate by waves or tidal currents. Physical removal from substrate during times of the year when there is no growth or ability to strengthen a weakened attachment is one possible cause of winter mortality in areas north of Cape Hatteras. For a soft-bodied suspension feeder such as *Styela*, removal from substrate is very likely equivalent to death. Simpson (1976) has observed that synergistic effects of several physical and biological factors limit the vertical distributions of intertidal molluscs. Similarly, it is likely that several factors together are responsible for the disappearance of *Styela plicata* at Cape Hatteras.

The capacity of an organism to regulate oxygen consumption is inversely related to the critical P_{O_2} . Physiological variables influencing the critical P_{O_2} are the gas diffusion distance, the rate of exchange of fluids on either side of the gas exchange membrane (the ventilation-perfusion ratio), and the respiratory surface area (SA) per unit volume of oxygen consumed (SA/V_{O_2}). Changes in any of these will affect the critical P_{O_2} .

The inverse relationship between P_c and body size of *Styela plicata* shown in Table II can arise if gas exchange area increases faster with increasing body size than does oxygen consumption, i.e., if SA/V_{O_2} increases with increasing body size. While increased ventilation/perfusion or a decreased diffusion distance can decrease P_c with increasing body size, the evidence described below suggests that increased respiratory surface area per unit volume of oxygen consumed, is at least partly responsible.

Pelseneer (1935) has reported that respiratory surface area and body size of adult molluscs form a constant ratio, which implies that the scaling factor relating respiratory surface area and body size is $b=1$. However, the scaling factor relating oxygen consumption of molluscs to body size is less than one (Ghiretti, 1966). This suggests that the ratio of respiratory surface area to oxygen consumed increases with increasing size. von Brand, Nolan, and Mann (1948) report a constant ratio of oxygen consumption to surface area of pulmonate and operculate snails. However, surface area was estimated as $W^{2/3}$, which does not necessarily predict respiratory surface area (SA). Therefore, it appears that respiratory SA/V_{O_2} increases with increasing body size of molluscs.

Although mammalian respiratory surface area is scaled in proportion to oxygen consumption, i.e., respiratory SA/V_{O_2} is constant (Tenney and Remmers, 1963), the weight dependence of respiratory SA/V_{O_2} of molluscs may be related to the dual functions of the molluscan respiratory pump: respiration and either filter-feeding or propulsion. Krogh (1951) and Wilbur and Yonge (1964) indicate that the gills of filter-feeding molluscs seem to be primarily adapted to their feeding habits, and the allometry between gill surface area and body size in molluscs may be a consequence of the physiological requirements of filter-feeding or propulsion, which apparently exceed the requirements for respiration. If this is also true for filter-feeding tunicates, then the critical oxygen tension could be expected to decline as a function of body size because of the increased respiratory surface area available for gas exchange.

The greater reduction in critical oxygen tension with increasing temperature may be the result of the increased rate of diffusion and/or a decreased diffusion distance. Since the activity of the ciliary pump in the branchial sac of *Styela plicata* can be expected to increase with temperature, a reduction in the water

boundary layer associated with the branchial sac should occur. In addition to the enhanced rate of diffusion, the relationship shown in Figure 3 may reflect a diminishing diffusion distance for oxygen with increasing temperature.

Opposite effects of temperature on critical oxygen tension have been observed in fish (Hughes, 1964; Graham, 1949). Rahn (1966) has emphasized the respiratory significance of the high ventilation rates of water breathers, but the relative ventilation rates of fish are generally less than one liter per ml O_2 consumed. Many invertebrate suspension feeders such as *Styela plicata* ventilate at much higher rates, up to 20 l/ml O_2 (Jørgensen, 1966, 1975), and the higher pumping rates of suspension feeders may be responsible for the different effects of temperature on the critical oxygen tension.

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SUMMARY

1. The oxygen consumption of the solitary tunicate *Styela plicata* was measured in order to estimate routine metabolic maintenance costs of the animal throughout the year.

2. The acclimatized oxygen consumption of *Styela* is proportional to the 0.7 power of body weight; this value is independent of the acclimatization temperature.

3. Q_{10} declines with increasing temperature, averaging 3 between 10° and 20° C, and 1.7 between 20° and 30° C.

4. Disproportionately large metabolic costs of routine activity cannot be invoked to explain the apparent lack of reproduction by *Styela plicata* during the warmest summer months.

5. The northern limit of *Styela plicata* is in the vicinity of Cape Hatteras, North Carolina. Winter mortality of adults is likely to limit the northern extension of *Styela* beyond Hatteras, and dislodgement from substrate during cold (growth inhibited) periods is suggested as one cause of winter mortality.

6. At temperatures greater than 10° C, oxygen uptake of *Styela* is independent of oxygen tension at normoxic conditions. An analysis of the critical oxygen tension as a function of temperature and body size suggests that ciliary activity may decrease the oxygen diffusion distance in the branchial sac at increased temperatures, and that the surface area per unit volume oxygen consumed may increase with increasing body size because of the demands of filter-feeding on the branchial sac.

LITERATURE CITED

- ANON., 1954. *World atlas of sea surface temperatures*. USN Hydrographic Office Publication No. 225.
- BAYNE, B. L., 1971. Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. *Comp. Biochem. Physiol.*, **40A**: 955-970.
- CERAME-VIVAS, M. J. AND I. E. GRAY, 1966. The distributional pattern of benthic invertebrates of the continental shelf off North Carolina. *Ecology*, **47**: 260-270.
- FISHER, T. R. 1976. The cost of metabolic maintenance of suspension feeding organisms. *Mar. Biol.*, in press.

- GHIRETTI, F., 1966. Respiration. Pages 175-208 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Molluscs*. Academic Press, New York.
- GRAHAM, J. M., 1949. Effects of temperature, oxygen pressure, and activity on metabolism of trout. *Can. J. Res.*, **27**: 270-288.
- HEMMINGSSEN, A. M., 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. *Reports Steno Mem. Hosp. Nordisk*, **9(II)**: 1-110.
- HUGHES, G. M., 1964. Fish respiratory homeostasis. *Soc. Exp. Biol. Symp.*, **18**: 81-107.
- JØRGENSEN, C. B., 1952. On the relationship between water transport and food requirements in some marine filter feeding invertebrates. *Biol. Bull.*, **103**: 356-363.
- JØRGENSEN, C. B., 1966. *The biology of suspension feeding*. Pergamon Press, New York, 357 pps.
- JØRGENSEN, C. B., 1975. Comparative physiology of suspension feeding. *Ann. Rev. Physiol.*, **37**: 57-79.
- KAZIHARA, T., 1964. Ecological studies on marine fouling animals. *Bull. Fac. Fish Nagasaka Univ.*, **16**: 1-138.
- KINNE, O., 1963. The effects of temperature and salinity on marine and brackish water animals. I. Temperature. *Oceanogr. Mar. Biol. Ann. Rev.*, **1**: 301-340.
- KROGH, A., 1941. *The comparative physiology of respiratory mechanisms*. University of Pennsylvania Press, Philadelphia, 172 pp.
- LEFEVRE, M. E., H. R. WYSSBROD, and W. A. BRONSKY, 1970. Problems in the measurement of tissue respiration with the oxygen electrode. *Bioscience*, **20**: 761-764.
- NEWELL, R. C., 1970. *Biology of intertidal animals*. Logos, London, 555 pp.
- PELSENEER, P., 1935. *Essai d'ethologie zoologique d'après l'étude des molluscs*. Acad. Roy. Belg. Classe. Sci. Publ. Fondation, Agathon de Potter, Brussels, 662 pp.
- RAHN, H., 1966. Aquatic gas exchange: theory. *Resp. Physiol.*, **1**: 1-12.
- SABBADIN, A., 1957. Il ciclo biologico di *Ciona intestinalis* (L.), *Molgula manhattensis* (de-Kay), e *Styela plicata* (Leseur) nella Laguna Veneta. *Archo. Oceanogr. Limnol.*, **11**: 1-28.
- SIMPSON, R. D., 1976. Physical and biotic factors limiting the distribution and abundance of littoral molluscs on MacQuarie Island (sub-antarctic). *J. Exp. Mar. Biol. Ecol.*, **21**: 11-49.
- TAYLOR, A. C. AND A. R. BRAND, 1975. Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica*. *J. Exp. Mar. Biol. Ecol.*, **19**: 187-196.
- TENNEY, S. M., AND J. E. REMMERS, 1963. Comparative quantitative morphology of the mammalian lung: diffusing area. *Nature*, **197**: 54-56.
- VAN NAME, W. G., 1945. The North & South American Ascidians. *Amer. Mus. Natur. Hist. Bull.*, **5**: 84.
- VON BRAND, T., M. O. NOLAN, AND E. R. MANN, 1948. Observations on the respiration of *Australorbis glabratus* and some other aquatic snails. *Biol. Bull.*, **95**: 199-213.
- WILBUR, K. M., AND C. M. YONGE, Eds., 1964. *Physiology of molluscs*. Academic Press, New York.